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RECOGNITION OF SYMBIOTIC ALGAE BY *HYDRA VIRIDIS*. A QUANTITATIVE STUDY OF THE UPTAKE OF LIVING ALGAE BY APOSYMBIOTIC *H. VIRIDIS*

ROSEVELT L. PARDY[1] AND LEONARD MUSCATINE

Department of Zoology, University of California, Los Angeles, California 90024

Many invertebrates form stable hereditary associations with unicellular algae. Experimental studies of these associations have dealt generally with the nutritional relationship between algae and host, and specifically with the translocation of carbohydrate from algae to animal (Smith, Muscatine, and Lewis, 1969). There have been very few experimental studies which describe how such associations are established and how the host recognizes potential symbiotic algae. Previous studies have been concerned mainly with specificity, *i.e.*, whether or not a given alga and host can form a stable hereditary symbiosis rather than with actual mechanisms of reinfection. (Whitney, 1907; Goetsch, 1924; Park, Greenblatt, Mattern and Merrill, 1967; Karakashian and Karakashian, 1964; 1965; Bomford, 1965; Hirshon, 1969; Provasoli, Yamasu, and Manton, 1968).

Specimens of *Hydra viridis* have *Chlorella*-like green algae which are hereditary endosymbionts. This symbiotic association offers several advantages for the study of host-symbiont recognition. The hydra can be reared in a defined medium. Asexual budding gives rise to large numbers of animals of similar genetic, nutritional and developmental histories. The algae, which are found in the hydra's endoderm and exclusively in the digestive cells, may be readily separated from the host cells and aposymbiotic hydra may be easily acquired, cultured, and experimentally recombined with algae.

In this paper we describe uptake of symbiotic algae by *Hydra viridis*, the rejection of free-living algae by these hydra, and the events leading to reestablishment of a stable association.

Materials and Methods

Maintenance and culture of organisms

Green specimens of *Hydra viridis* (Florida strain 61) were cultured in "M" solution (Muscatine and Lenhoff, 1965a) under ambient laboratory conditions 22° C, 30 foot candles, 14 hours light and 10 hours dark, and fed daily on freshly hatched *Artemia* nauplii. *H. viridis* occasionally produces algae free eggs. From these eggs we developed a class of aposymbiotic hydra, which we cultured and maintained as above. Algae strains and their sources are listed in Table I. All algal stocks with the exception of *H. viridis* symbionts, were maintained in 1.5% agar slants of Loefer medium (LMM) as modified by Karakashian (1963). Sub-cul-

[1] Present address: Department of Developmental and Cell Biology, University of California, Irvine, California 92664.

TABLE I

Summary of algae used in this study

Strain	Host	Remarks
3H	*Paramecium bursaria*	Isolated and cultured by Karakashian (1963)*
34	*Paramecium bursaria*	Isolated and cultured by Karakashian (1963)*
130C	*Paramecium bursaria*	Isolated and cultured by Loefer (1936)**
42A	*Paramecium bursaria*	Isolated and cultured by Karakashian (1963)*
NC64A	*Paramecium bursaria*	Isolated and cultured by Karakashian (1963)*
838	*Spongilla* sp.	Isolated and cultured by R. A. Lewin**
397	Free-living *Chlorella vulgaris*	**
	Algae from *Hydra viridis*	Isolated en masse from cultures of the host

* Obtained from Dr. Stephen J. Karakashian, State University of New York, College of Old Westbury.

** Obtained from the Indiana University Culture Collection of Algae.

tures of algae were prepared aseptically as needed in 5 ml liquid LMM in 15 ml test tubes at 22° C under the continuous illumination of two 40-watt fluorescent lights situated 15 cm below the bottom of the culture tubes. Five days after inoculation these subcultures yielded approximately 0.15 ml wet packed algae (*ca.* 10^8 cells per ml). Symbionts from *H. viridis* were isolated from about 400 one-day starved hydra as described by Muscatine (1967). This procedure yielded about 6×10^7 algae which were used immediately in experiments.

Preparation of ^{14}C-labeled algae

Algae harvested from hydra or obtained from cultures were incubated for 45 min, in 1 ml of "M" solution containing 5 μCi $NaH^{14}CO_3$. Labeled algae were then washed 3 times in "M" solution and an aliquot of cells was withdrawn for assay of radioactivity. Such incubation yielded algae with a relative specific activity of 7×10^5 disintegrations min^{-1} mg^{-1} protein nitrogen. Protein nitrogen was measured by the method of Lowry, Rosebrough, Farr and Randall (1951).

Injection of algae into hydra

The injection apparatus consisted of 0.1 ml capacity Hamilton glass syringe fitted with heavy-walled, 2 mm inside diameter Tygon tubing. The syringe and tubing were filled with mineral oil. The injection pipette was prepared from glass capillary tubing drawn out to approximately 0.15 mm outside diameter at the tip. Cell sus-

pensions (10^7–10^8 cells per ml) were introduced into the pipette and the cells were allowed to settle in the tip. The filled pipette was then inserted into the Tygon tubing, taking care to avoid introduction of air bubbles. At the same time, slight suction was applied with the syringe to prevent algae from being prematurely expelled from the pipette. Before injection, the base of each relaxed, attached hydra was touched with the tip of the pipette causing the hydra to retract into a spherical shape. The tip of the pipette could then be easily inserted into the mouth of the hydra. Once the tip was introduced into the mouth it was held in place for a few seconds to allow the hydra's mouth to close tightly around it. The thick algal suspension was then delivered by slight pressure on the syringe plunger. With practice the injection volume was adjusted so that it inflated the hydra to roughly twice its original size. While it was not always possible to control or even measure the volume of algae injected, more cells were injected than could be taken up by the hydra. Over-inflation was avoided since it caused immediate collapse of the hydra and regurgitation of the algae, regardless of the type of algae injected. Further, when the pipette was removed, the mouth invariably gaped and algae tended to leak out into the medium. To prevent gaping, hydra to be injected were each fed a single *Artemia* nauplius and then injected within a few minutes after the shrimp was swallowed.

Assay of radioactivity in hydra and algae

To measure the quantity of labeled algae taken up by hydra digestive cells, individual injected animals were placed in a drop of "M" solution and slit down one side with a scalpel. Algae not taken up by the digestive cells were flushed from the interior with a fine pipette. The washed hydra and the solution with unincorporated algae were each applied to separate half-inch squares of Whatman 3 mm filter paper, treated with two drops of 0.1 N HCl to remove unused $^{14}CO_2$ and dried under a heat lamp. The dried filter paper squares were assayed for radioactivity in 5 ml scintillation fluid (50 g 2,5-diphenyloxazole, 0.625 g 1,4-bis, 2-5 phenyloxazolyl benzene in 500 ml toluene) using a Nuclear Chicago liquid scintillation counter (Model *Mark I*). Uptake of algae into hydra digestive cells was expressed as a percentage of the total radioactivity in algal cells administered by injection. The possibility existed that soluble ^{14}C-labeled material might move from the algae in the coelenteron to the hydra cells even though the algae themselves might not be taken up. To check this possibility an injection was performed using algae labeled with $Na_2{}^{35}SO_4$. The uptake pattern of these algae did not differ significantly from those labeled with $NaH^{14}C_3$ which was used in all other determinations. In some experiments, whole green hydra and aposymbiotic hydra were incubated in 1 ml "M" solution containing 5 μCi $NaH^{14}CO_3$ for one hour under the continuous illumination of "Gro-Lux" fluorescent lamps delivering about 260 footcandles to the surface of the hydra. Following incubation, the hydra were rinsed, dried on Whatman 3 mm squares, treated with 0.1 N HCl, and counted as described above.

Maceration of hydra

To examine individual hydra digestive cells for the presence of algae and to count their numbers, hydra tissue was macerated on a microscope slide in either

FIGURE 1. Photomicrograph of a typical green hydra digestive cell obtained by maceration and showing the basal lower location of the symbiotic green algae which number 25 in this cell (A, algal cells; N, nucleus of the digestive cell), 936 ×, Nomarski interference microscopy.

0.2% acetic acid and then fixed with a drop of 0.004% osmium tetroxide or a solution consisting of acetic acid, glycerol, and water (1:1:13 v/v) and observed immediately, under a compound microscope at 400 ×. Counts were recorded with a hand tally counter.

RESULTS

To describe the uptake of algae by aposymbiotic hydra, it was necessary first to characterize quantitatively the normal population of algae in green hydra. Three criteria were used: (a) total number of algae per hydra, (b) mean number of algae per digestive cell, and (c) amount of ^{14}C fixed by whole green hydra, shown previously to be proportional to the number of algae present (Muscatine and Lenhoff, 1965b).

Total number of algae in green hydra

Each of ten individual hydra, one-day starved and having one bud, were homogenized with a small tissue grinder in 0.5 ml "M" solution. Aliquots of this

TABLE II

Effect of maintenance conditions on the number of algae per digestive cell in H. viridis. *Animals were maintained at room temperature. Illumination (200 ft-c) was provided by Sylvania Gro-lux lamps. Diurnal room illumination was 10 hrs light (30 ft-c) and 14 hrs dark. Data expressed as average number of algae/digestive cell ± s.d.; N = 100 cells from Zone 2 of five hydra*

Condition	Number of algae	
	Day 2	Day 4
Constant light, starvation	21.0 ± 5.7	21.8 ± 4.6
Constant light, fed every 24 hr	20.0 ± 4.7	20.8 ± 4.1
Diurnal light, starvation	20.4 ± 1.0	20.0 ± 1.7
Diurnal light, fed every 24 hr	18.0 ± 2.6	18.3 ± 1.9
Constant dark, starvation	17.0 ± 1.4	11.0 ± 3.0
Constant dark, fed every 24 hr	12.3 ± 3.8	6.8 ± 2.3

homogenate were transferred to a hemacytometer and from the algal count the number of algae per hydra was obtained. Such hydra were found to contain $1.47 \times 10^5 \pm 4 \times 10^4$ algal cells.

Number of algae per digestive cell

Gross examination of whole green hydra revealed that the densest population occurred in the mid-zone of the polyp rather than in the tentacles or stalk. To quantify this distribution, individual hydra were cut transversely into three pieces; hypostome and tentacles (Zone 1); gastric column including the budding region (Zone 2); and the stalk and pedal disc (Zone 3). Pieces from each zone were macerated separately on a microscope slide and the intracellular algae in individual digestive cells were counted. The greatest mean number of algae per digestive cell was observed in cells from Zone 2. In this zone about 18 ± 2.6 algae were encountered in each cell most frequently. Significantly fewer algae per cell (approximately 12) were encountered in Zones 1 and 3. These data show that the algae are distributed throughout the body but the central region contains the highest number of algae per cell.

Figure 1 shows a *Hydra viridis* digestive cell prepared by maceration. The zoochlorellae are located in the proximal portion of the cell. In normal green hydra the algae were always found in this position in digestive cells and never in any other cell types.

It is important to note here that in experiments to be described elsewhere, we found that maintenance of hydra under different light and feeding regimes re-

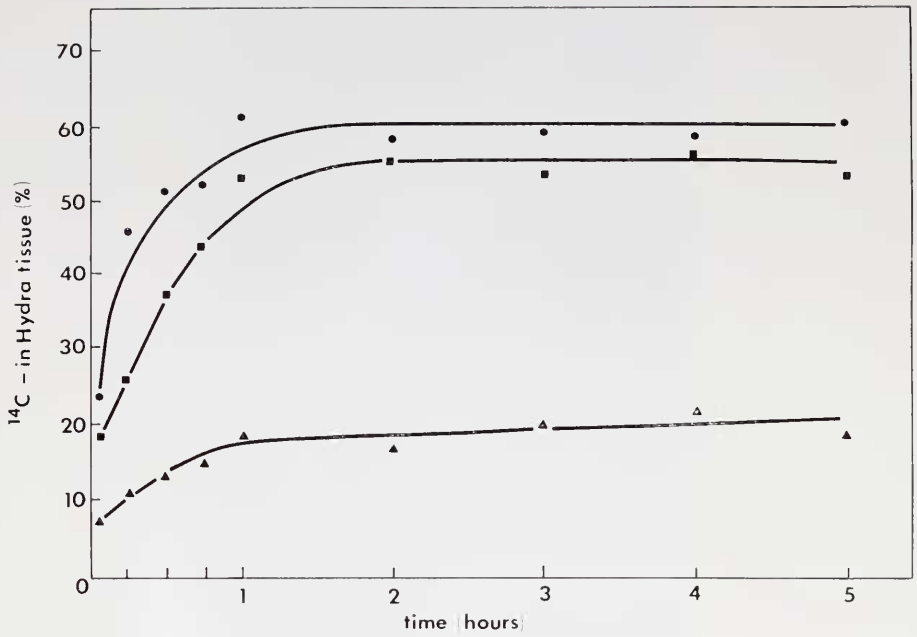

FIGURE 2. Per cent of radioactivity in hydra tissue following time after injection of ^{14}C-labeled algae (open circle, aposymbiotic hydra injected with hydra algae; open square, green hydra injected with hydra algae; open triangle, aposymbiotic hydra injected with NC64A algae; curves fitted by eye).

sulted in different mean numbers of algae per digestive cell, ranging from 7 to 22 as shown in Table II. For example, animals fed daily but kept in darkness had far fewer algae per cell than a hydra fed daily but kept in constant light. Presumably, a hydra fed in darkness grows well enough but its algae do not. We chose to use animals which came from populations fed daily and maintained under ambient diurnal light conditions (30 footcandles, 14 hours light, 10 hours dark). However, data in this paper apply only to animals maintained as described in Methods.

Uptake of algae by aposymbiotic H. viridis

Algae were isolated from normal *H. viridis* and then injected into aposymbiotic *H. viridis*. At various times after injection the hydra were assayed for radioactivity to determine the extent of uptake of algae. Figure 2 shows that the uptake of the radioactively labeled algae is very rapid. Half of the total ^{14}C incorporated by the hydra is taken up in the first two minutes following injection. Uptake of algae by digestive cells continues more slowly thereafter for about one hour and then ceases even though excess algae are still abundant in the coelenteron.

To observe the location and fate of algae after phagocytosis by digestive cells, aposymbiotic hydra were macerated at 30 seconds, 10 minutes, 30 minutes, and finally after 5 hours after injection. Figure 3a shows initial contact of algae and

digestive cell. Figure 3b shows that algae are taken up at the distal portion of the cell. About 30 minutes after injection, algae can be found throughout the digestive cell interior, as shown in Figure 3c. Finally after 5 hours, the algae are generally located at the base of the cell, as in normal green hydra digestive cells (3d).

About 5 hours after feeding, *H. viridis* normally regurgitates particulate material not taken up by digestive cells. After regurgitation, the number of algae taken up by aposymbiotic *H. viridis* was determined by maceration and cell counts. Digestive cells of Zone 2 had taken up about $8 \pm 2 (n = 100)$ algae per digestive cell. Since we had previously observed that normal green hydra have 18 ± 2.6 algae per digestive cell (Zone 2), we reasoned that the normal complement of 18 must be obtained by algal cell division. This possibility was investigated by following the course of repopulation.

Repopulation of aposymbiotic H. viridis

Aposymbiotic hydra were injected with unlabeled hydra algae. Five hours later, following regurgitation, and at various times thereafter, the hydra were incubated in $NaH^{14}CO_3$ for one hour and then assayed for fixed ^{14}C. The data in Table III show that the amount of ^{14}C fixed increases most rapidly during the first 168 hours after uptake of algae, and then more slowly until after about 18 days, the amount of ^{14}C fixed is similar to that fixed by green controls. Digestive cells observed at that time had 19 ± 3 algae per cell indicating that repopulation was complete. During the time that repopulation was taking place, the injected hydra fed and produced buds normally. We conclude that following injection and uptake of algae by *H. viridis* the algae undergo relatively rapid cell division until the full complement of algae is obtained.

Uptake of algae by normal green H. viridis

The foregoing data demonstrate that, even if given excess algae, the digestive cells of aposymbiotic *H. viridis* take up only about half of their normal complement of algae, the remainder being acquired by cell division. The data suggest, therefore, that the limitation on uptake of algae is not lack of physical space since ultimately the hydra cells can accommodate 19 algae and in some cases (Fig. 1) as many as 25 per digestive cell. This conclusion was further tested by injecting *H. viridis* algae into normal green hydra. Since the algae injected into hydra were labeled with ^{14}C, they were distinguishable from algae already present in the digestive cells. The curve in Figure 2 shows that green hydra possess the capacity to take up additional algae. Uptake was slower than that of aposymbiotic hydra; half the labeled algae being taken up in about 7 minutes. Digestive cells obtained 5 hours after injection possessed approximately 27 ± 4.9 algae per cell (Zone 2) $(n = 100)$. Since normal green hydra have approximately 18–19 algae per cell, it is evident that roughly 9 extra algae were taken up by digestive cells following injection of algae. This is the approximate number which were taken up by aposymbiotic hydra. The subsequent fate of these extra algae has not yet been determined.

Injection of foreign algae into aposymbiotic H. viridis *and other* Hydra *spp.*

We attempted to infect *H. viridis* aposymbionts and other *Hydra* species with various species of foreign algae, both symbiotic and free-living. Six strains of

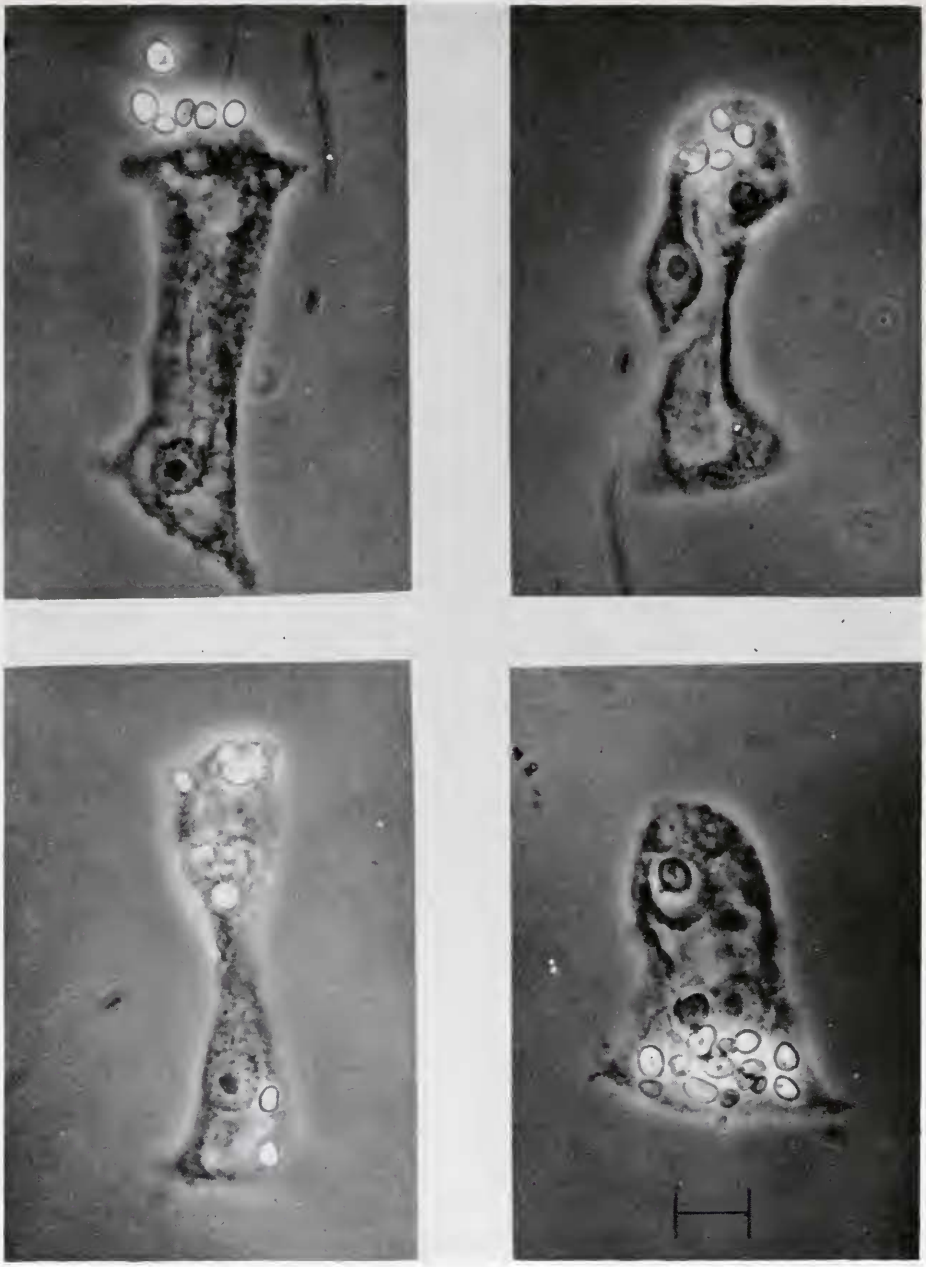

FIGURE 3. Phase contrast photomicrographs showing digestive cells macerated from aposymbiotic hydra at various times after injection of hydra algae. Phase optics employed to distinguish algae (+ birefringence) from other cell organelles and inclusions. Cells oriented with distal absorptive ends up (N, nucleus of animal cell; A, algal cells); starting at upper left, (a) 30 sec after injection, showing algae in contact with distal portion of digestive cell (note

TABLE III

Radioactivity (disintegrations min^{-1}) in whole hydra due to $^{14}CO_2$ fixation by intracellular algae at various times after injection with normal hydra algae. Counts are the average of three hydra. Green control shows the level of $^{14}CO_2$ incorporated by algae in standard green hydra. Non-injected aposymbiotic control indicates the level of heterotrophic fixation of $^{14}CO_2$ by hydra

Time after injections	Disintegrations min^{-1}
0.2 (days)	91
1	110
2	145
3	147
6	164
7	179
18	210
32	203
H. viridis (control)	218
Aposymbiotic hydra	15

symbiotic algae, five from *P. bursaria* and one from *Spongilla* sp. and one species of free-living *Chlorella* were tested. Injection of *H. viridis* algae into aposymbiotic hydra served as a control. Success of infection was determined from observations on macerated cells and ability to fix $^{14}CO_2$. The results in Table IV indicate that of the algae injected, only algae from *H. viridis* and one strain of algae from *P. bursaria* were taken up by aposymbiotic *H. viridis*. All other strains of algae were regurgitated almost immediately (5–10 minutes) by some individuals, and in all cases within 5 hours after injection. Those algal strains taken up by *H. viridis* aposymbionts were also injected in nonsymbiotic hydra. There was no evidence of uptake of these algae by cells from these animals (*H. littoralis* or *H. pseudoligactis*). Figure 2 shows that the rate of uptake of NC64A algae by *H. viridis* is slower than that of *H. viridis* algae, with half of the total ^{14}C incorporated being taken up in 10 minutes.

We have found that those aposymbiotic hydra injected with NC64A algae have slightly fewer algae per cell (approximately 15) 60 days after injection than aposymbiotic hydra controls 30 days after injection (approximately 20). Figure 4 shows that the growth rate of hydra infected with NC64A is virtually identical with that of normal green hydra under identical conditions of feeding. We concluded from these data that NC64A algae can establish a successful symbiosis with *H. viridis* although the rate of uptake of the algae, the rate of algal cell repopulation, and the final population density is less than those of normal hydra algae.

DISCUSSION

The results of these experiments show that aposymbiotic *Hydra viridis* can be reinfected after receiving an injection of its normal symbiotic algae or one strain of

flagellum); (b) 10 min, algae are now within the digestive cell as a result of phagocytosis; (c) 30 min, location of algae shifting from distal to proximal (basal) region; (d) 5 hr, Algae now in normal basal location (*cf.* Fig. 2). Scale bar represents 10 microns.

TABLE IV

Comparison of various algae in their ability to be taken up by specimens of aposymbiotic H. viridis; (−) *not taken up,* (+) *taken up*

Algal strain	Host	H. viridis
311	*Paramecium bursaria*	−
34	*Paramecium bursaria*	−
130c	*Paramecium bursaria*	−
42	*Paramecium bursaria*	−
NC64A	*Paramecium bursaria*	+
838	*Spongilla* sp.	−
hydra	*Hydra viridis*	+
397	Free-living *C. vulgaris*	−

(symbiotic) algae from *Paramecium bursaria*. On the other hand, aposymbiotic *H. viridis* rejects other strains of *P. bursaria* algae, sponge algae, and free-living algae. These observations, together with the fact that *H. pseudoligactis* and *H. littoralis* reject symbiotic algae (NC64A, *H. viridis* algae) suggest that the establishment of symbiosis in aposymbiotic *H. viridis* is a function of properties of both the algae and the hydra and that the discrimination between them leading to either uptake or rejection may be referred to legitimately as a cellular recognition phenomenon.

Four general phases may be distinguished in the successful establishment of the *H. viridis* symbiosis: (1) A brief period of *contact* and *recognition* followed by (2) *phagocytosis* which may last up to one hour, (3) intracellular transport lasting at least five hours, and finally, (4) a period of algal *growth*, initially relatively rapid, lasting up to two weeks.

Aposymbiotic *H. viridis* becomes reinfected only when the algae are introduced into the colenteron (Goetsch, 1924; Whitney, 1907; Park *et al.*, 1967), presumably because contact between algae and digestive cells is required to initiate the recognition mechanism. We tentatively rule out such physiological parameters as oxygen production by the algae as a significant feature of the recognition mechanism leading to acceptance since all algae produce oxygen but most are still rejected. Similarly, the release of soluble extracellular carbohydrate by symbiotic algae (Smith, *et al.*, 1969) may be ruled out as a chemical cue leading to recognition since all but one of the symbionts were rejected. It would be difficult to explain any specificity in the recognition mechanism based on oxygen production or release of carbohydrate. Current models of cell recognition are based generally on the interaction of molecules at cell surfaces (Smith and Good, 1969). In this connection we note that Slautterback (1967) has detected and described "coated vesicles" in the apical cytoplasm of digestive cells of *Hydra oligactis*. These vesicles are involved in selective phagocytosis of particulate material. They are coated with a highly ordered array of electron dense "pegs" and "globules." As such they emerge as a morphological entity worthy of consideration as possible "recognition sites," that is contingent, of course, on whether or not they occur in *H. viridis*.

Data in Figure 2 show that when the algae are recognized as acceptable symbionts, their uptake into digestive cells is relatively rapid, the process being nearly

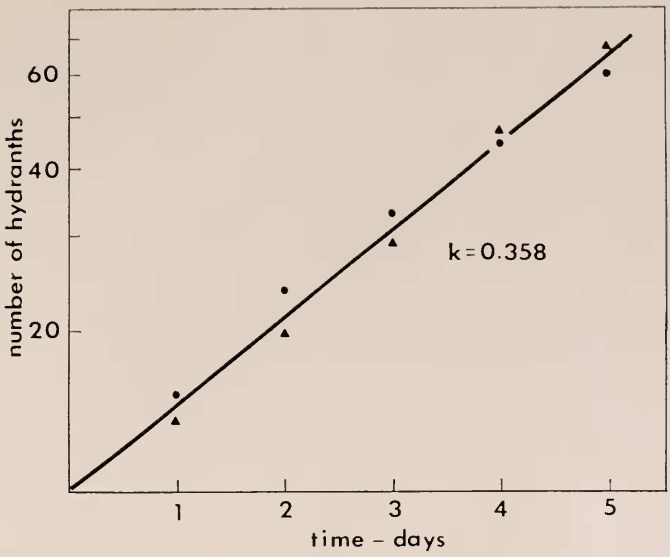

FIGURE 4. Growth rate of green hydra (open triangle with normal algal flora and aposymbiotic hydra artificially infected with NC64A algae (open circle).

completed in less than an hour. These uptake rates compare favorably with those reported for the uptake of *Chlorella* by *P. bursaria* (Hirshon, 1969). It is evident that uptake of algae is accomplished by phagocytosis and results in the formation of intracellular vacuoles but the exact mechanism has not yet been determined. Symbiont vacuoles are described in studies on the fine structure of green hydra by Wood (1959), Oschman (1967), and Park *et al.* (1967). In *Paramecium bursaria* the route of infection is uncertain but algae are believed to be ingested into large vacuoles resembling food vacuoles. At this point foreign algae are sorted out and expelled and symbiotic algae are retained in inidividual vacuoles (Karakashian and Karakashian, 1965; Karakashian, Karakashian and Rudzinska, 1968). When symbiotic algae undergo cell division, their vacuole may temporarily contain several autospores, but these are ultimately disposed into new individual vacuoles (Oschman, 1967; Karakashian *et al.*, 1968).

Of the several factors which may limit the uptake of algae by *H. viridis* to 8 ± 2 cells, the supply of algae and the space available for them can be ruled out on the basis of our observations. Rather, the limiting factor is probably the phagocytosis process itself. The metabolic basis of phagocytosis is fairly well understood. It is temperature and pH dependent and requires energy. Lipid and protein synthesis are required for the formation of vacuolar membranes. (Rowley, 1962; Karnovsky, 1962; Jacques, 1969). We speculate therefore that uptake of algae is limited by the ability of the cells to synthesize vacuolar membranes. The extent to which the presence of food in the coelenteron influences the recognition and uptake of algae has not yet been investigated in detail. Our unpublished observations suggest that the presence of food is not required for uptake of algae but food may compete for phagocytic vacuole membranes which might otherwise be used to

take up algae. Neither the stimulus for intracellular transport of algae, its mechanism, nor its selective advantage is yet known.

Little is known of the population dynamics of symbiotic algae. It is generally conceded that the growth rates of algae and host are somehow linked to keep the symbiont population at an optimum level. Experiments in our laboratory suggest that algal growth rates and standing crops may be modified temporarily by environmental factors such as light and nutrients (see also Oschman, 1967; Weis, 1969). For example, green hydra fed in darkness outgrow their algae to the extent that there are only a few algae per digestive cell (Table II). When these hydra are returned to the light the algae immediately undergo rapid growth and within a few days the population is established at an optimum level. Similarly, after injection of algae, aposymbiotic hydra have relatively few algae per cell. Table III shows that within a few days the algae undergo relatively rapid growth until the full complement of algae is attained. These data show that the ability of the symbionts to grow at high maximum intrinsic rates is important in maintaining the population at an optimum level. The factors which contribute to the onset and cessation of these rapid algal growth rates are still unknown.

Associated with the problem of growth is the problem of distribution of algae within a given host. We assume that because we supply excess algae to aposymbiotic hydra, that all digestive cells obtain at least a few symbionts. This is not necessarily true. In fact, small numbers of algae injected into an aposymbiotic hydra would satisfy only a few digestive cells. How do the rest of the digestive cells acquire symbionts from this initial small inoculation? There are at least three possibilities. (1) The algae move from cell to cell. This appears unlikely especially in view of the observation of Burnett and Garofalo (1960) of the fate of bits of green hydra tissue grafted onto albino hydra. (2) The algae are expelled into the coelenteron and then immediately recaptured by other digestive cells. This possibility has not yet been experimentally tested. (3) The remaining aposymbiotic cells do not become infected, rather the rate of cell division of green (algae-laden) digestive cells is faster than aposymbiotic cells and the green cell line becomes dominant as all cells undergo their normal turnover in the hydra. This alternative is consistent with the fact that green hydra grow faster than aposymbiotic hydra particularly when food is limiting (Muscatine and Lenhoff, 1965b).

The rejection of foreign algae by *H. viridis* is also intriguing. Unlike *P. bursaria* where the rejection of foreign algae is perhaps decided after the algae are within a vacuole, rejection by algae by *H. viridis* is apparently decided at the cell surface and leads to a coordinated response involving the whole hydra. Not only is mucus secreted locally to bind the foreign algae together but increased flagellar and muscular activity is elicited and in some cases the algae are gathered up and pushed toward the hypostome by movements of the body in preparation for expulsion only minutes after they are injected into the coelenteron. The ecological importance of rejection should not be underestimated. It is highly likely that in nature *Hydra* feeds on herbivorous crustaceans which themselves have recently ingested unicellular algae. The result is that foreign algae are introduced into their enterons when the crustaceans are digested. The fate of such algae is unknown but the fact that foreign algae are rejected argues against the likelihood that hydra routinely digests algae. Karkashian *et al.* (1968) argue that part of the success-

ful maintenance of algae inside cells prone to autophagy may be their ability to resist lysosomal digestive activity. The algal vacuoles in *Paramecium bursaria* were not observed in association with lysosomes. In contrast, Oschman (1966) has observed "Phagolysosomes" fusing with algae vacuoles. He interprets this to mean that *Hydra* probably digests its algae either wholly or in part under appropriate circumstances. Despite inferences from electron microscopy, conclusive evidence for digestion of algae (*i.e.*, enzymatic hydrolysis of algal consituents) is still lacking.

Infection of aposymbiotic hydra with foreign algae has been reported by Goetsch (1924) and Park *et al.* (1967). In each case the free-living alga *Oocystis* was taken up by the aposymbiotic *H. viridis*. However, in both instances the infection was transitory and the algal population did not reach the level normally found in green hydra. We did not attempt to reinfect *H. viridis* with *Oocystis* and tested only one species of free-living algae which was rejected, and so we regard any conclusions about specificity of infection of *H. viridis* as tentative. However, the infection of *H. viridis* by strain NC64A from *P. bursaria* showed no tendency to decline even though the population densities were slightly less than those in normal green hydra. It thus appears that a persistent symbiosis between *Hydra viridis* (Florida strain 61) and NC64A can be attained. Since NC64A is not a natural symbiont of Hydra, it is not unexpected that its kinetics of uptake and growth rate are different from those of normal symbionts.

The re-establishment of a symbiosis with algae is not unique to *Hydra*. Trench (1969) has shown that aposymbiotic *Anthopleura elegantissima,* a sea anemone, may be re-infected by repeated injection of their normal dinoflagellate symbionts. Provasoli *et al.* (1968) has brought about the re-synthesis of algal symbiosis in *Convoluta roscoffensis*, an acoel flatworm. *Paramecium bursaria* can acquire and maintain other algal strains as well as its native symbionts (Bomford, 1965; Karakashian and Karakashian, 1965; Hirshon, 1969) but in most cases the native symbionts exhibit greater persistence and survival in the host.

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Summary

1. An individual *Hydra viridis* (Florida Strain) harbors approximately 1.5×10^5 unicellular green algae. These algae are normally found in the basal portion of gastrodermal digestive cells. An average of approximately 18 algae per digestive cell is encountered in the central region (stomach and budding zone) of the hydra under a given set of maintenance conditions.

2. The average number of algae per digestive cell may range from 7 to 22 depending on feeding schedules and photoperiod under which hydra cultures are maintained.

3. The symbiosis can be synthesized artifically by injecting symbiotic algae from *H. viridis* into the coelenteron of aposymbiotic *H. viridis*. Within 15 minutes after injection the algae are taken up by the digestive cells, apparently by phagocytosis. During the next hour, the algae move from the site of uptake to the basal part of the digestive cell.

4. Both green and aposymbiotic *H. viridis* can take up only about 8 ± 2 algae from an injected suspension of cells. Uptake may be limited by the extent to which the cell can sustain phagocytosis, rather than by availability of intracellular space. In aposymbiotic *H. viridis*, the full complement of 18 algae is attained by algal cell division and requires about 18 days.

5. An assortment of free-living and symbiotic algae were injected into *H. viridis* aposymbionts. All were rejected immediately with the exception of hydra algae and a symbiotic alga from *Paramecium* (NC64A) which formed a stable hereditary endosymbiosis with *H. viridis*. Growth rates of these hydra were virtually identical with those of normal green hydra.

6. The acquisition of algae by hydra digestive cells appears to involve several "phases" including contact, recognition, phagocytosis, and intracellular transport.

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